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ORIENTATION IN THE BENZENE RING: THE PREPARATION OF 5-CHLORO-β-RESORCYLIC ACID.

and

STUDIES IN THE STRUCTURE OF SOME FLUORESCEIN COMPOUNDS.

BY

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A THESIS

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Edmonton, Alberta. April, 1935.

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ORIENTATION IN THE BENZENE RING. THE PREPARATION OF 5-CHLORO-β-RESORCYLIC ACID.

INTRODUCTION.

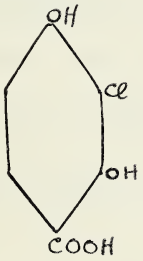
In 1922, Fabre[1] published the results of his study of the constitution of resorcinol and some of its derivatives, in which he outlined the preparation of two chloro-β-resorcylic acids, 3-chloro-2,4-dihydroxy benzoic acid, and 3-chloro-2,6-dihydroxy benzoic acid. He prepared the first acid by treating β-resorcylic acid in ethereal solution with sulfuryl chloride, and obtained needle-shaped crystals which when dried melted at 203°C. The second acid was prepared by refluxing 4-chlororesorcinol with potassium bicarbonate for one and one-half hours in water solution. This compound when dried melted at 215-216°C., and was reported as being less soluble than the first acid.

Moreover, in 1926, Hemmelmeyr and Meyer[2] in their study of the effect of different substituents on the stability of carboxyl groups in substituted aromatic acids, reported the preparation of a third

chloro-β-resorcylic acid, which they called 5-chloro-2,4-dihydroxy benzoic acid. This was prepared by the direct chlorination of β-resorcylic acid in hot glacial acetic acid solution, and melted at 175°C.

Thus the preparation of the following three chloro-β-resorcylic acids has been reported in the literature:

(1)



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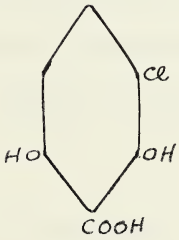
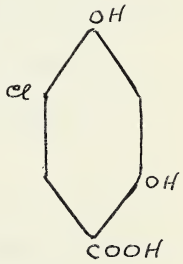


(3)



OUTLINE OF PRESENT INVESTIGATION.

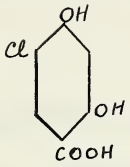
It was decided to repeat the preparation of these three compounds and to characterize them by the preparation of their monoacetates according to the method of Lesser and Gad.[3] To characterize these compounds by melting points alone is not conclusive proof of their nature since the melting points of certain compounds of resorcinol are very liable to variation. In this regard it may be noted that β-resorcylic acid itself has been recorded in the literature as having at least seven different melting

points.[4]

Thus a far more conclusive proof of the character of these three acids would be to prepare thdir monoacetates and then determine the mixed melting points of these acetates. This was done, and so far as can be determined the three acetates are the same, and therefore the three chloro-β-resorcylic acids are the same, and correspond to Hemmelmeyr and Meyer's 5-chloro-2,4-dihydroxy benzoic acid, only with a higher melting point.

That it is the 5-chloro-2,4-dihydroxy benzoic acid is shown by the work of Lesser and Gad, who in their investigation of the acetylation of aromatic hydroxycarboxylic acids showed that aromatic hydroxycarboxylic acids which do not contain the hydroxyl group in ortho position to the carboxyl radical are readily and quantitatively acetylated by the addition of a slight excess of acetic anhydride to the acid dissolved in aqueous alkali. Thus it can be concluded from the formation of a monoacetate in the case where the acid is prepared from the action of potassium

bicarbonate on 4-chlororesorcinol, that the carboxyl group in that acid must be in ortho and para position with respect to the two hydroxyl groups, and consequently, since all the acids are identical:

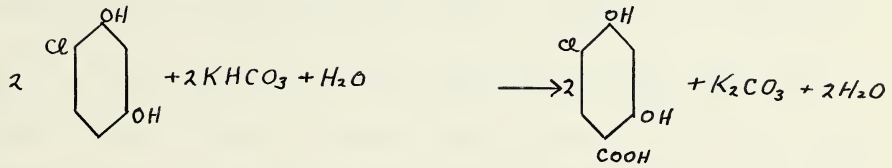


is the acid formed in all three methods of preparation.

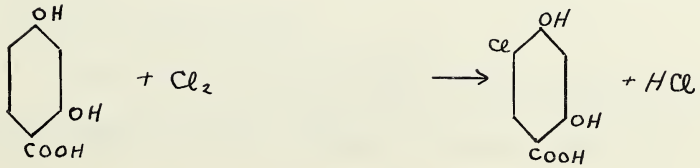
The equations involved in the three different reactions above are as follows:

METHOD (1).

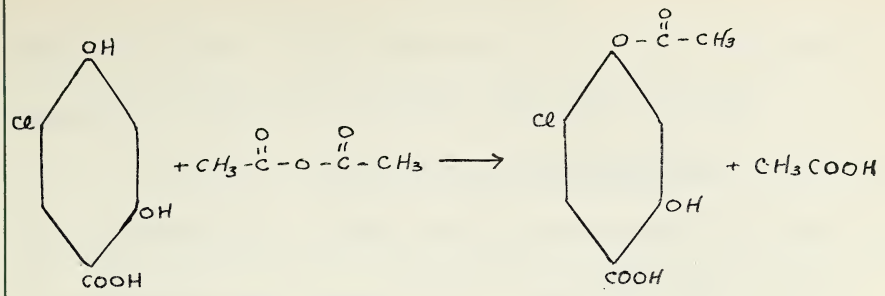
METHOD (2).



METHOD (3).



and for the formation of the monoacetate:



EXPERIMENTAL PART.

PREPARATION OF 5-CHLORO-β-RESORCYLIC ACID AND ITS MONOACETATE.

METHOD (1).

30.8 g. (.2 mole) of β-resorcylic acid was warmed in 200 cc. of glacial acetic acid solution with stirring until it was all dissolved. 27.0 g. (.2 mole) of sulfuryl chloride dissolved in 200 cc. of glacial acetic acid was added dropwise with vigorous stirring to the warm solution over a period of about one hour. On completion, the reaction mixture was poured into four litres of water and cooled in the ice-chest overnight. The crude acid was filtered off and dried, and the yield was 19 g.

The crude product was recrystallized a number of times from boiling water and dried at 100° C. for four hours, until it had a constant melting point of

224-5°C.(corr.) with decomposition. This compound on analysis showed the correct chlorine content.

10.0 g. of the acid was dissolved in 100 cc. of approximately normal sodium hydroxide solution, and the solution was cooled in an ice-bath. 15. g. of acetic anhydride was added dropwise with mechanical stirring to the cold solution over a period of one-half hour. After standing a few minutes 20. cc. of concentrated hydrochloric acid was added. The crude product was filtered off and washed with water. The acetate was recrystallized eight times from 50% ethyl alcohol, 100 cc. of water being added to the filtrate after filtering, until it had a constant melting point of 169°C. (corr.)

ANALYSIS: Calc. for $C_9H_7O_5Cl$: Cl: 15.40

Found Cl: 15.30, 15.46

METHOD (2).

PREPARATION OF 4-CHLORORESORCINOL:

This was prepared according to the method of Reinhard.[5] 22.0 g. (.2 mole) of resorcinol was dissolved in 100 cc. of ether, the solution being kept

in an ice-bath. 13.5 g. (.1 mole) of sulfuryl chloride was added dropwise to the cold solution with vigorous stirring over a period of one-half hour. On completion, the ether was first removed by distillation, then the 4-chlororesorcinol was distilled off. The boiling point was 258-9°C. and the yield was 21 g.

The monochloro-β-resorcylic acid was prepared according to the method of Nierenstein and Clibbens.[6] In a 500 cc. flask fitted with a reflux condenser, 21. g. of 4-chlororesorcinol, 105. g. of potassium bicarbonate, and 210 cc. of water were heated slowly on a steam bath for four hours. The flask was then placed over a flame and the solution refluxed vigorously for thirty minutes while a rapid stream of carbon dioxide was passed through. While still hot, the solution was acidified with 95 cc. of concentrated hydrochloric acid by means of a separatory funnel reaching to the bottom of the flask. The solution was thoroughly cooled and the monochloro-β-resorcylic acid was filtered off. The acid was recrystallized from water and after filtering was placed

in an ice-bath to promote rapid recrystallization. The yield was 5 g., and the acid had a melting point of 214°C. with decomposition.

Five g. of the acid was converted into the monoacetate in the manner described in METHOD (1). The acetate was recrystallized nine times from 50% ethyl alcohol, 100 cc. of water being added to the filtrate after filtering, until it had a constant melting point of 169°C. (corr.)

ANALYSIS: Calc. for $C_9H_7O_5Cl$: Cl: 15.40

Found Cl: 15.37, 15.38

METHOD (3).

Three hundred and fifty cc. of glacial acetic acid was saturated in the dark with 23.05 g. (.33 mole) of chlorine from a chlorine generator. Fifty g. (.33 mole) of β-resorcylic acid was warmed in 200 cc. of glacial acetic acid with stirring until it was all in solution. The chlorine solution was then added dropwise with vigorous stirring to the β-resorcylic acid solution over a period of two and one-half hours, this reaction also being carried out

in the dark. The reaction mixture was poured into a large evaporating dish and allowed to evaporate at room temperature. The crude yield was 71 g. The crude product was refluxed for two hours with 400 cc. of water to remove any of the dichloro compound which may have formed,[7] hot filtered, and cooled. The melting point was 199-207°C. (corr.) with decomposition.

Five g. of the acid was converted into the monoacetate in the manner described in METHOD (1). The acetate was recrystallized eight times from 50% ethyl alcohol, 100 cc. of water being added to the filtrate after filtering, until it had a constant melting point of 169°C. (corr.)

ANALYSIS: Calc. for $C_9H_7O_5Cl$: Cl: 15.40

Found Cl: 15.30, 15.38

Mixed melting point determinations were run on equal mixtures of samples from methods (1) and (2), (1) and (3), (2) and (3), and finally (1), (2), and (3), and in no case was there any depression of the melting point recorded. Therefore it can be concluded from the above work that the three acids are identical, and

that it is the 5-chloro-2,4-dihydroxy benzoic acid which has been prepared in each case.

SUMMARY.

The preparation of 5-chloro-2,4-dihydroxy benzoic acid by three different methods has been described and the identical nature of the three acids obtained has been discussed.

NOTE:-

In connection with this investigation, the monoacetates of monobromo- and dibromo-, monoiodo- and diiodo-, and monochloro- and dichloro-β-resorcylic acid were prepared in an attempt to determine whether all these monoacetates could be used for characterization purposes. Repeated recrystallization of the monoacetates was carried out in each case until they had constant melting points, but only in the case of the monohalogenated compounds did the monoacetates give the same melting point over a series of two or more preparations, as can be seen from the accompanying table.

This fact would seem to show that there is some limitation to the use of melting points of the monoacetates, as prepared by the method of Lesser and Gad, for characterization purposes, in the case of dihalogenated derivatives of β-resorcylic acid. An analysis was run on all of the monoacetates, and they showed in every instance the correct halogen content.

It is possible that this apparent discrepancy in the case of the dihalogenated compounds can be explained by the fact that the dihalogenated compounds are much more unstable than the monohalogenated compounds, losing carbon dioxide much more readily on being boiled with water. Kharasch, Reinmuth, and Mayo [8] have pointed out that dibromo-β-resorcylic acid loses 84.7% carbon dioxide on boiling in water for one hour, whereas the monobromo-β-resorcylic acid loses only 19.0% carbon dioxide on boiling in water for the same length of time. They also state that β-resorcylic acid itself loses 49.1% carbon dioxide on boiling in water for one hour, hence we arrive at the conclusion that one halogen substituent in the β-res-

orcylic acid molecule tends to stabilize the carboxyl group, while two halogen substituents tend to make the carboxyl group more unstable. In a series of preparations of the dibromo-β-resorcylic acid therefore, it would be barely possible to get a monoacetate with a constant melting point when the parent substance can be decomposed so readily. Similar circumstances exist in the case of the diiodo- and the dichloro- derivatives. There is reason to believe however, that the ease of decomposition of these dihalogenated derivatives is not the whole cause of the variation of the melting points of the monoacetates, but that the problem is much more complex.

EXPERIMENTAL.

Preparation of the monoacetate of 5-bromo-2,4-dihydroxy benzoic acid:

15.4 g. (.1 mole) of β-resorcylic acid was warmed in 100 cc. of glacial acetic acid with stirring until it was all in solution. Sixteen g. (.1 mole) of bromine, dissolved in 80 cc. of glacial acetic acid, was added dropwise to the warm solution over a period

of one hour with vigorous stirring. The reaction mixture, a yellow solution, was then poured into two litres of water and cooled for one hour. The crude product was filtered off and washed with water, giving a crude yield of 20 g. The crude product was refluxed for one hour with 500 cc. of water to remove any dibromo compound that may have been formed, hot filtered, cooled, and filtered. The purified yield was 14 g. and the melting point was 206-8°C. (corr.)

The monoacetate was prepared by the method of Lesser and Gad, and was recrystallized six times from 50% ethyl alcohol, until it had a constant melting point. (See Table 1).

Preparation of the monoacetate of 3,5-dibromo-2,4-dihydroxy benzoic acid:

15.4 g. (.1 mole) of β-resorcylic acid was brominated in the same manner as in the case of the monobromo compound above, 32 g. (.2 mole) of bromine being used in this case. The crude yield was 21 g. The acid was recrystallized from 50% ethyl alcohol, water being added to the filtrate after filtering, and dried

at 100°C. for six hours. The yield was 16 g.

The monoacetate was prepared by the method of Lesser and Gad, and was recrystallized six times from 50% ethyl alcohol, using animal charcoal to remove color once or twice, until it had a constant melting point. (See Table 1).

Preparation of the monoacetate of 5-iodo-2,4-dihydroxy benzoic acid:

15.4 g. (.1 mole) of β-resorcylic acid was warmed in 100 cc. of glacial acetic acid with stirring until it was all in solution. 8.1 g. (.05 mole) of iodine monochloride dissolved in 100 cc. of glacial acetic acid was added dropwise to the warm solution with vigorous stirring over a period of one hour. The reaction product was poured into two litres of water and cooled for one hour. It was then filtered and dried. The crude yield was 13 g. The crude acid was used for the preparation of the monoacetate.

The monoacetate was prepared by the method of Lesser and Gad, and was recrystallized ten times from

50% ethyl alcohol, until it had a constant melting point. (See Table 1).

Preparation of the monoacetate of 3,5-diiodo-2,4-dihydroxy benzoic acid:

15.4 g. (.1 mole) of β-resorcylic acid was iodinated in the same manner as in the case of the monoiodo compound, 32.5 g. (.2 mole) of iodine monochloride being used in this case. The crude acid resulting was recrystallized three times from 75% acetone. The yield was 21 g.

The monoacetate was prepared by the method of Lesser and Gad, and was recrystallized ten times from 50% ethyl alcohol, using animal charcoal for the first five recrystallizations to remove the gummy character of the compound, until it had a constant melting point. (See Table 1).

Preparation of the monoacetate of 5-chloro-2,4-dihydroxy benzoic acid:

The preparation of this monoacetate has already been fully described in the main portion of the

investigation outlined above. (For the constant melting point see Table 1).

Preparation of the monoacetate of 3,5-dichloro-2,4-dihydroxy benzoic acid:

15.4 g (.1 mole) of β-resorcylic acid was chlorinated in the same manner as in the case of the monochloro compound, 27.0 g. (.2 mole) of sulfuryl chloride being used in this case. The acid was purified before the preparation of the monoacetate.

The monoacetate was prepared by the method of Lesser and Gad, and was recrystallized eight times from 50% ethyl alcohol, until it had a constant melting point. (See Table 1).

(Table 1 on next page.)

TABLE (1).

MONOACETATE:	PREP. (1). M.P.(corr.).	PREP. (2). M.P.(corr.).
(1) Monobromo	156°C.	157°C.
(2) Dibromo	137°C.	195°C.
(3) Monoiodo	137°C.	188°C.
(4) Diiodo	203°C.	214°C.
(5) Monochloro	169°C.	169°C.
(6) Dichloro	184°C.	187°C.

ANALYSIS:

(1) Calc. for $C_9H_7O_5Br$: Br: 29.07

Found Br: 29.01 , 29.01

(2) Calc. for $C_9H_6O_5Br_2$: Br: 44.88

Found Br: 45.02 , 45.15

(3) Calc. for $C_9H_7O_5I$: I: 39.30

Found I: 39.30 , 39.07

(4) Calc. for $C_9H_6O_5I_2$: I: 56.7

Found I: 57.1 , 56.4

(5) Calc. for $C_9H_7O_5Cl$: Cl: 15.40

Found Cl: 15.30 , 15.46

(6) Calc. for $C_9H_6O_5Cl_2$: Cl: 26.79

Found Cl: 26.76 , 26.83 , 26.65

PART TWO.

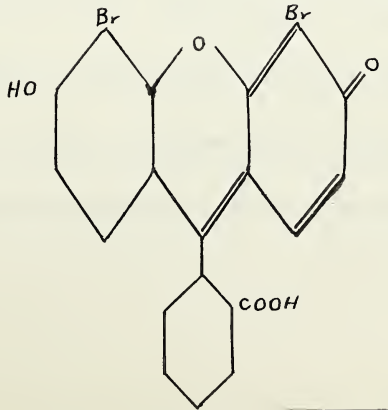
STUDIES IN THE STRUCTURE OF SOME FLUORESCEIN COMPOUNDS.

INTRODUCTION.

The compound fluorescein was first prepared by Baeyer [9] in 1876 during his investigation of the compounds resulting from the reaction of phthalic acid with different phenols. In this work he reported the preparation of a dibromofluorescein, which he prepared by the direct bromination of fluorescein in glacial acetic acid solution, using two moles of bromine to one of fluorescein. Baeyer did not give a structural formula for his dibromofluorescein, but reported it as forming compact reddish-brown needles with a deep green reflection, having a melting point of 260-270°C. On refluxing with acetic anhydride this compound gave a diacetate crystallizing in colorless needles, which melted at 208-210°C. Phillips [10] says that it is doubtful if this dibromofluorescein was a pure compound, or indeed any dibromofluorescein prepared in this manner

in the presence of solvents.

Heller and Meyer,[11] in 1909, stated that Baeyer's asymmetric dibromofluorescein could best be prepared by heating a mixture of dibromodihydroxybenzoylbenzoic acid and resorcinol in molecular proportions with zinc chloride at 170°C. This compound crystallized from alcohol in dark red prisms with solvent of crystallisation, and was reported as melting at 300°C. The diacetate crystallized from toluene with solvent of crystallization in plates melting at 173°C. with decomposition. Orndorff and Hemmer,[12] in 1927, were the first investigators to report the preparation of pure dibromofluorescein, which they prepared by fractional crystallization and hydrolysis of the dibenzoate, and which had a melting point of 280-290°C. with decomposition. They gave as the structure of this compound:

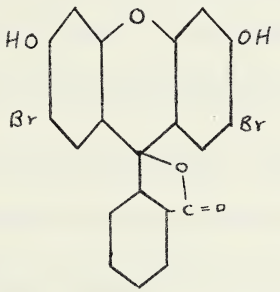


believing that fluorescein can exist in two forms, the yellow lactoid form and the red quinoid form, and that the colored dibromofluorescein is a derivative of the quinoid fluorescein. The dibenzoate of this dibromofluorescein was prepared and was found to melt at 239-240°C. It was colorless and was a derivative of the lactoid fluorescein. No diacetate was reported.

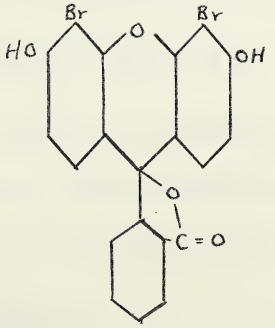
Phillips, mentioned above, in 1932 outlined the preparation of a pure dibromofluorescein which he prepared by the action of bromine on fluorescein in 80% acetic acid solution, and which gave a melting point of 285°C. He found that bromination as carried out by Baeyer leads to variable mixtures of dibromofluorescein hydrobromide and fluorescein, and pure dibromofluorescein hydrobromide could not be obtained. However, with the use of 80% acetic acid as solvent, he obtained an 85 - 88% yield of the dibromofluorescein hydrobromide, from which, by the action of hot water to quantitatively remove the combined hydrogen bromide, pure dibromofluorescein was obtained. This compound

gave a diacetate which crystallized from acetic anhydride or nitrobenzene in plates and had a melting point of 211°C.

In the older textbooks on organic chemistry, the structural formula of dibromofluorescein was written with the bromine atoms in the 2,7 positions as follows:



This would seem to be the most probable structure for dibromofluorescein, but in recent years the structure has been altered so that the bromine atoms go into the 4,5 positions in the fluorescein molecule, as follows:



It has been thoroughly established, according

to Hewitt and Woodforde,[13] that the 4,5 positions in the fluorescein molecule are invariably the first in which substitution occurs. They carried out an alkali fusion on dinitrofluorescein and obtained 2-nitro-resorcinol, similar to that obtained by Matras,[14] thus showing that the substitution of the nitro groups takes place at the 4,5 positions in the fluorescein molecule. They further prepared 2,7-dibromo-4,5-dinitrofluorescein by brominating dinitrofluorescein, and also 4,5-dibromo-2,7-dinitrofluorescein by the nitration of dibromo-fluorescein, and found these two compounds to be isomers. Holmes, Melin, and Peterson [15] have carried this work further and have determined the absorption spectra of the two isomeric dibromodinitrofluoresceins and have found these to be different. Though these compounds are admittedly dissimilar, the positions of the substituent groups is determined from the results of the alkali fusions, only, and it is not believed that this evidence is conclusive as to the positions of the substituent groups.

OUTLINE OF PRESENT INVESTIGATION.

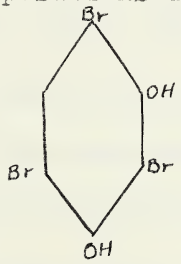
It would seem to be desirable from the above discussion therefore, to attempt to prove definitely whether the substitution of the bromine atoms in dibromofluorescein takes place in the 4,5 positions or in the 2,7 positions, and this was one of the main objectives of this research. It would also be desirable to know, if possible, what was the underlying cause of such widely divergent results obtained by different workers in the preparation of dibromofluorescein. This work on the structure of dibromofluorescein is of fundamental importance, because of the marked and promising bactericidal properties of some of its derivatives, especially mercurochrome, which is a mercurated derivative of dibromofluorescein. It would be advantageous also to know whether there was only one dibromofluorescein, in which the bromine atoms were in the 4,5 positions, or whether there existed as well, an isomer, in which the bromine atoms were in the 2,7 positions, and hence to be able to determine the relative bactericidal action of the two isomers.

The problem was attacked first by an attempt to prepare pure dibromofluorescein from 4-bromoresorcinol. If the dibromo compound could be prepared this way, we would be sure that the bromine atoms were in the 2,7 positions in the fluorescein molecule, and from a comparison of the absorption spectra of this compound with the absorption spectra of the dibromofluorescein prepared by the direct bromination of fluorescein, it would be possible to tell whether the two compounds were the same or were isomers of one another. If they were the same, then the fact stated by Hewitt and Woodforde that substitution takes place primarily in the 4,5 positions in the fluorescein molecule would be disproved. If they were different, then it would be necessary to determine the physical and chemical properties of the new isomer, the 2,7-dibromofluorescein.

To carry out this work, it was first necessary to prepare 4-bromoresorcinol. A method of preparation has been developed in this laboratory which is essentially a modification of that described in Beilstein.[16] Other methods[17] have been outlined for the preparation

of 4-bromoresorcinol, but these lack simplicity and seem to have led to varying degrees of success. The method to be described give a good yield of practically pure 4-bromoresorcinol, and eliminates much of the detail of other procedures.

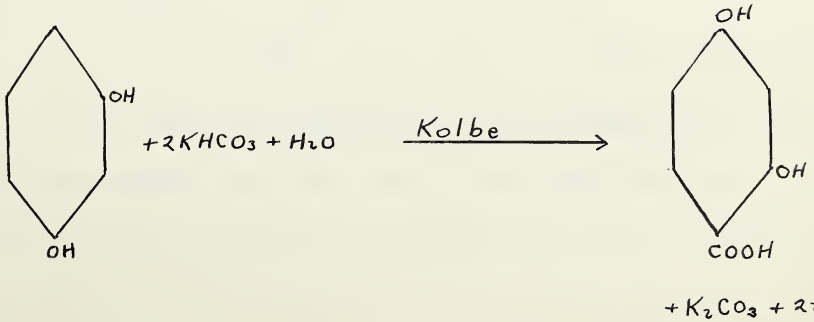
It is known that if resorcinol is brominated directly, the bromine atoms will go into the resorcinol molecule in the 2,4,6 positions as follows:

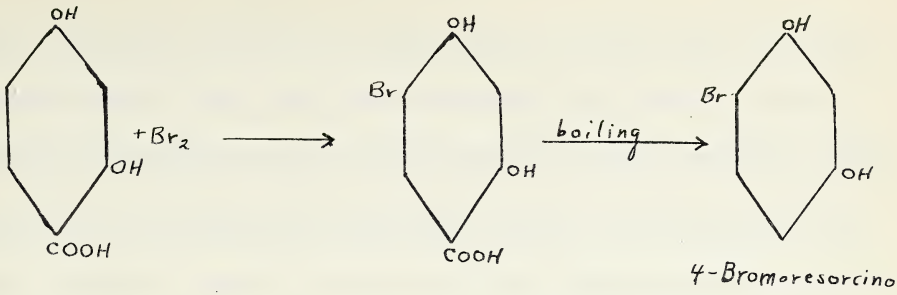


to give tribromoresorcinol. However, if a carboxyl group is first substituted in the ortho para position with respect to the two hydroxyl groups, on bromination with the calculated quantity of bromine, the 5-bromo-2,4-dihdroxy benzoic acid is obtained, and possibly some of the 3,5-dibromo-2,4-dihydroxy benzoic acid. This latter compound can be readily removed by boiling in water for one hour when the dibromo compound is practically all decomposed into dibromoresorcinol

which is very soluble in cold water.[18] The monobromo dihydroxy benzoic acid is then further refluxed for twenty-four hours with water to remove the carboxyl group, leaving the 4-bromoresorcinol which is also soluble in water. This 4-bromoresorcinol is extracted twice with ether and the ether evaporated to give a compound melting at 100-2°C. In some cases a compound is obtained from the evaporation which has a melting point ranging from 72 - 95°C., but this can be changed, by dissolving in hot chloroform and evaporating off the chloroform, into the compound melting at 100-2°C. This latter process is not believed to be a purification, although if necessary the 4-bromoresorcinol can be recrystallized from chloroform, but with a large loss in yield.

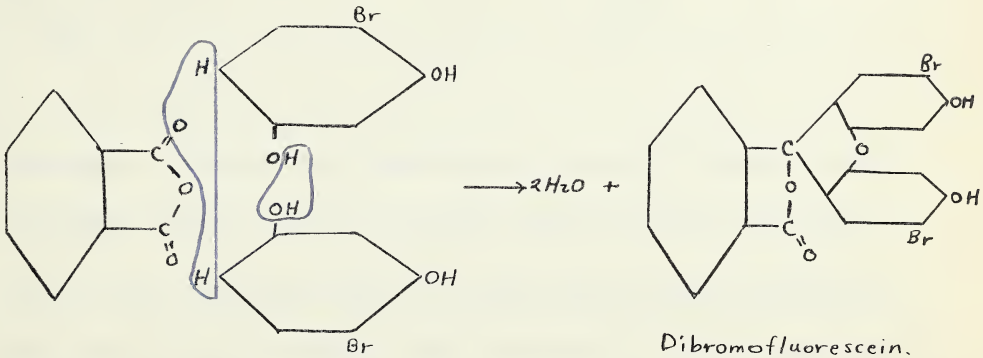
The reactions involved in the above preparation are as follows:





4-Bromoresorcinol.

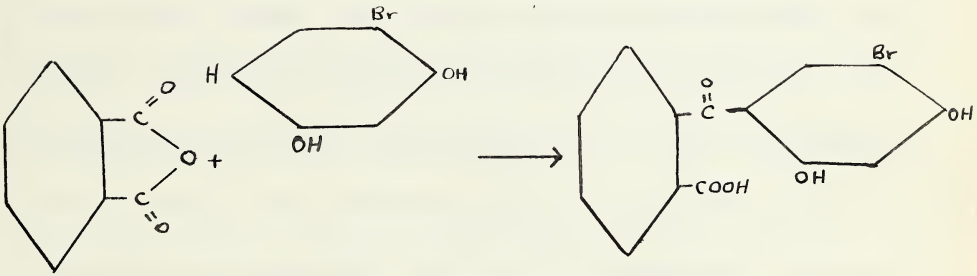
To prepare dibromofluorescein from this 4-bromoresorcinol two moles of the 4-bromoresorcinol were fused with one mole of phthalic anhydride at a temperature of 190°C. in the presence of anhydrous zinc chloride, until the mass was hard and glassy. The fusion product was ground, dissolved in sodium bicarbonate, filtered, and acidified with dilute hydrochloric acid, to give the dibromofluorescein. The reaction is as follows:



Dibromofluorescein.

So far the attempts at the preparation of dibromofluorescein by this method have met with poor success. It is obviously necessary to obtain a very

pure compound before the spectrophotometer can be used, and as yet it has not been possible to obtain a pure dibromofluorescein by this method, so the deciding evidence of the absorption spectra cannot be obtained. It was thought possible that the poor results were due to the use of too low a temperature for the fusion with phthalic anhydride and the resulting formation of an intermediate monobromodihydroxybenzoylbenzoic acid as follows:



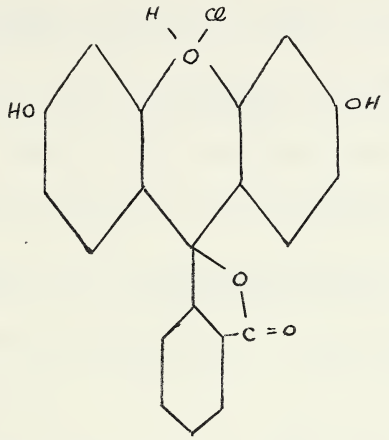
However, a fusion was carried out using a higher temperature (about 210°C.) and the results were the same as in the previous case. It was also thought possible that the time of fusion was too short, but it was found that increasing the time likewise had no effect on the product.

The problem has been attacked therefore from a different viewpoint, and it has been found necessary to rely on the results of alkali fusions far more than would have been desired. Pure dibromofluorescein has been prepared by Phillip's method and also by a method making use of an oxonium salt formation. Phillips reported the preparation of dibromofluorescein by the addition of the calculated quantity of bromine to fluorescein in 80% acetic acid as solvent, but it was found that unless the bromine solution was added practically instantaneously the resulting product did not possess the correct melting point. If the bromine was added slowly, the melting point was lowered, and the derivatives prepared, such as the diacetate and the dibenzoate, did not give constant values for melting points or for analysis. However, when the bromine was added rapidly, which seemed contrary to the best procedure, a pure dibromofluorescein was obtained melting at 283-285°C. Further difficulty was encountered in the preparation of the diacetate of this compound. A diacetate was obtained from the above dibromofluorescein

that melted at 229°C., which checked with the melting point of the diacetate from the oxonium salt preparation to be described, but which did not check with Phillips' reported diacetate, which melted at 211°C. It is believed that Phillips failed to recrystallize his diacetate enough, since he reported using acetic anhydride as the solvent, and acetic anhydride is much too good a solvent, even in the cold, to be used for many recrystallizations.

Dibromofluorescein was also prepared by a method making use of the formation of an oxonium salt of fluorescein, then brominating with the calculated quantity of bromine. Fischer and Bollemann[19] have reported the preparation of oxonium salts of fluorescein by adding alcohol to a heated mixture of powdered fluorescein and concentrated hydrochloric acid, or by passing hydrogen chloride into an ethereal solution of fluorescein. In the present preparation, a modification of the former method was used, in which the fluorescein was completely brought into solution in glacial acetic acid and concentrated hydrochloric acid

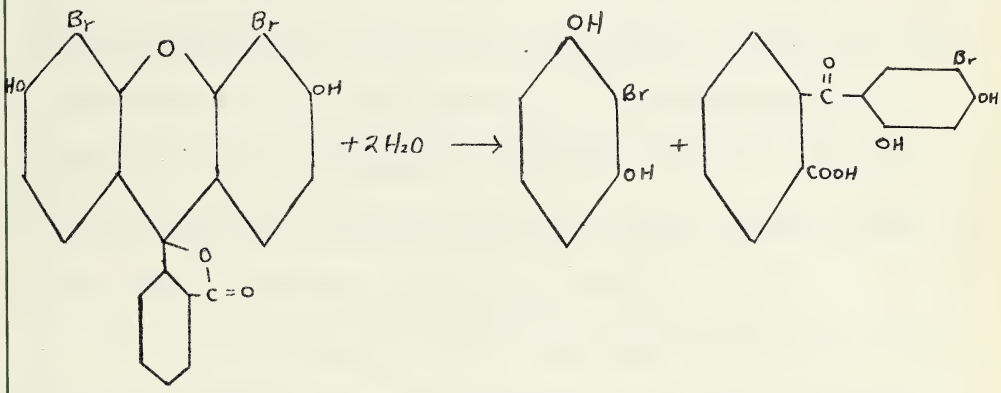
before bromination. This formed a clear yellow solution of the oxonium salt of fluorescein, with a structure as follows:



Bromination was then carried out slowly with the calculated amount of bromine, and when the reaction was about half complete, the solution became turbid with the formation of a red precipitate, which was the oxonium salt of dibromofluorescein. Oxonium salts are unstable and easily hydrolysed, so the salt was filtered off and boiled with a large quantity of water to hydrolyse it, then filtered off again and washed thoroughly with hot water to obtain the dibromofluorescein. This compound gave a diacetate which was exactly similar to the diacetate obtained from the direct bromination of fluorescein, and which gave on analysis the

correct bromine content. The diacetate was hydrolysed with 85% sulfuric acid, poured into water, filtered, and washed thoroughly with hot water to give a pure dibromofluorescein melting sharply at 235°C.

Alkali fusions were carried out on these dibromofluoresceins using a modification of Hewitt and Woodforde's method, to determine whether a monobromo resorcinol could be isolated from the fusion products. The reaction is:



Dibromofluorescein from the preparation using the method of Phillips, for example, was dissolved in 50% sodium hydroxide solution and refluxed for two hours. The reaction product was acidified with hydrochloric acid, extracted with benzene, and the benzene evaporated. It was found that the residue sublimed to some extent

even at room temperature, so the substance was sublimed on a hot-plate and condensed and recovered on the inside of a large beaker. This substance was very pure, was in the form of long needles, and had a melting point of 102.5°C. Grace Potter Rice [20] in some work which she undertook on the orientation of the bromine atom in bromo-dimethoxybenzoic acid, reports 2-bromoresorcinol as long needles having a melting point of 102.5°C., and further reports a mixture of 2-bromoresorcinol and 4-bromoresorcinol melting at 65 - 82°C. A mixed melting point was run on a 50% mixture of 4-bromoresorcinol, which had been previously prepared, with the unknown bromoresorcinol obtained from the sodium fusion, and this gave a melting point of 74-92°C.

From this it can be concluded that the fusion product must be 2-bromoresorcinol, and hence the dibromofluorescein prepared from the above procedures must have the bromine atoms in the 4,5 positions in the fluorescein molecule, in accord with the newer structure advanced for dibromofluorescein, and confirming the work of Hewitt and Woodforde. An alkali fusion

was run on some 4-bromoresorcinol in the same manner to make sure there was no rearrangement to form the 2-bromoresorcinol during the fusion, but no change could be detected. This is as far as the results of the alkali fusion will lead us, and we are forced to accept, like Hewitt and Woodforde, the evidence of the alkali fusion, to tell us in which position the bromine atoms are situated in the fluorescein molecule. Further work will be carried on in an attempt to prepare a dibromofluorescein in which the bromine atoms are in the 2,7 positions, if this compound can be prepared.

EXPERIMENTAL PART.

(1) PREPARATION OF 4-BROMORESORCINOL:

The purpose here was to develop a simple reliable method for the preparation of 4-bromoresorcinol, eliminating the involved technique of previous methods, and at the same time to evolve a method which would give uniform results and a pure product. Several trial runs

were made which have been tabled below, and the procedure outlined below was the outcome of the results of these trial runs. The preparation of this compound can properly be divided into two parts: (a) the formation of 5-bromo-2,4-dihydroxy benzoic acid, and (b) the removal of carbon dioxide to give 4-bromoresorcinol.

(a) The Preparation of 5-bromo-2,4-dihydroxy benzoic acid:

The procedure of the trial runs in general was briefly as follows: β-resorcylic acid [21] was dissolved in a solvent and then brominated by adding bromine dissolved in the same solvent. The reaction product was poured into water, cooled, filtered, and dried. Finally, the crude product was boiled for one hour to decompose any dibromo compound that may have formed, filtered, and dried.

(See Table (2) for results of trial runs.)

TABLE (2).

Results of Trial Runs:

15.4 g. of β-resorcylic acid and 16. g. of bromine were used in every case.

	Solvent used	cc. solvent used for β-resorcylic acid	cc. solvent used for bromine	Time of bromination
1.	glacial acetic acid	100	100	90 min.
2.	ether at 0°C.	100	100	90 "
3.	ether at 0°C.	100	100	90 "
4.	glacial acetic acid	100	100	90 "
5.	glacial acetic acid	50	50	90 "
6.	ether at 0°C.	100	100	60 "
7.	glacial acetic acid	100	100	60 "

	H_2O used	Crude yield	H_2O used for elimination of dibromo compound	Purified yield	Melting point.
1.	200 cc.	15 g.	400 cc.	10 gm.	199-200°C.
2.	Ether evapor.	--	400	--	--
3.	Ether evapor.	--	400	12	--
4.	2000 cc.	17	400 (charcoal)	7	--
5.	2000 cc.	13	400	8	--
6.	Ether evapor.	12	400	8	--
7.	2000 cc.	16.5	400	11	--

From the above results it was concluded that glacial acetic acid was the best solvent for the bromination, that the reaction product should be poured into a large excess of water, and that charcoal should not be used to remove color because it materially reduces the yield.

(b) The Preparation of 4-bromoresorcinol:

The procedure of the trial runs in general was briefly as follows: The 5-bromo-2,4-dihydroxy benzoic acid was boiled for twenty-four hours with different amounts of water, cooled, and filtered. Dilute sulfuric acid was added, then the solution was extracted three times with ether, the ether portion washed with dilute ammonium carbonate, the ether portion separated, and the ether finally evaporated to yield the 4-bromoresorcinol.

(See Table (3) for results of trial runs.)

TABLE (3).

Results of trial runs:

	Monobromo β-resorcylic acid used.	H_2O used	H_2SO_4 used	cc. portions ether used.
1.	36 g.	1800 cc.	250 cc.	500,300,200 cc.
2.	92	4600	700	1500,750,400
3.	14	100	100	200,100,100
4.	25	625	omitted	400,200,100
5.	25	625	omitted	400,200,100
6.	25	315	omitted	400,200,100

	$(NH_4)_2CO_3$ used	Yield	Melting point (uncorr.)	Recrystallized from $CHCl_3$ M. P. (uncorr.)
1.	1000 cc.	21 g.	84 - 91°C.	101 - 102°C.
2.	2600	49	101 - 102	101 - 102
3.	400	1	98 - 101	---
4.	omitted	17	99 - 102	101 - 102
5.	omitted	19	75 - 93	100 - 102
6.	omitted	19	77 - 93	100 - 102

It was found in the above runs that the time of refluxing could not be shortened, as a mixture of the monobromo β-resorcylic acid and 4-bromoresorcinol resulted which gave an impure product. In another run the ether portions from the three extractions were evaporated separately, and it was found that the yield

from the third extraction was practically nothing, so the third extraction was omitted as being of no value. Omitting the addition of sulfuric acid and also the washing with ammonium carbonate solution did not have any harmful effect on the purity of the product obtained. Twelve and one-half times the quantity of monobromo β-resorcylic acid used was found to be the optimum quantity of water to use for refluxing. Davis and Harrington [22] have reported that they could only obtain a 20% yield of 4-bromoresorcinol on boiling 5-bromo-2,4-dihydroxy benzoic acid with water for twenty-four hours, but we have had no difficulty in obtaining 90% yields of the pure 4-bromoresorcinol by our method.

From the results of the trial experiments above, a detailed procedure for the preparation of 4-bromoresorcinol can now be set up, which is simple, reliable, and which yields a pure product. It is as follows:

In a 1 litre Erlenmeyer flask fitted with a dropping funnel and mechanical stirrer 46.2 g. (.3 mole) β-resorcylic acid is dissolved in 300 cc. of glacial acetic acid by gently warming and stirring. 48. g. of

bromine is dissolved in 240 cc. of glacial acetic acid and placed in the dropping funnel. With vigorous stirring the bromine solution is allowed to drop very slowly into the β-resorcylic acid solution, the reaction taking place over one hour. The reaction mixture is poured into five litres of water and allowed to stand in the ice-chest one hour or longer. The monobromo β-resorcylic acid is filtered off and dried. Crude yield is 54 - 60 g. (77 - 85 per cent of the theoretical amount.)

The crude product is refluxed for one hour with 1500 cc. of water, hot filtered, cooled in an ice-chest, and the purified product filtered off and dried. The yield is 40 - 44 g. (57 - 63 per cent of the theoretical amount.) Melting point is 203-206°C. (uncorr.), of the air-dried material.

Thirty g. of pure monobromo β-resorcylic acid is refluxed for twenty-four hours with 375 cc. of water, and filtered. The solution is then extracted twice with ether using 200 and 100 cc. portions respectively. The ether portion is evaporated and the monobromo resorcinol

collected. The yield is 22 - 22.5 g. (90 - 92 per cent of the theoretical amount.) The melting point is 99-102°C. (uncorr.)

A great many runs have been made using this procedure, in order to check its reliability, and at the same time to obtain a good supply of 4-bromoresorcinol for future work. The results of these runs are tabled below:

TABLE (4).

Preparation of 5-bromo-2,4-dihydroxy benzoic acid:

	β-resorcylic acid used.	Br_2 used.	Crude yield.	Purified yield.	Melting Point (uncorr.)
1.	15.4 g.	16 g.	20. g.	14.5 g.	202-204°C.
2.	15.4	16.	18.	13.	200-201
3.	15.4	16.	20.	15.	201-203
4.	15.4	16.	19.	14.5	199-200
5.	15.4	16.	20.	13.5	199-200
6.	15.4	16.	18.5	14.	199-200
7.	15.4	16.	17.	13.	201-202
8.	15.4	16.	17.5	12.5	202-203
9.	15.4	16.	19.	14.	201-203
10.	15.4	16.	18.5	13.5	200-203
11.	15.4	16.	18.5	13.5	201-203
12.	30.8	32.	39.5	26.	201-202
13.	30.8	32.	34.	23.	201-203
14.	30.8	32.	35.	25.	202-204
15.	46.2	48.	58.	41.	204-205
16.	46.2	48.	59.	44.	204-205
17.	46.2	48.	60.	42.	205-206
18.	46.2	48.	54.	40.	204-205
19.	46.2	48.	55.	40.	204-205
20.	46.2	48.	58.	41.	203-204

An analysis was run on No. 3 above:

ANALYSIS: Calc.for $C_7H_5O_4Br$: Br: 34.32

Found Br: 34.33

TABLE (5).

Preparation of 4-bromoresorcinol:

	Monobromo β - resorcylic acid used.	Yield.	Melting point.
1.	30.g.	22.g.	100-102°C.
2.	30.	22.	100-102
3.	30.	22.5	99-102
4.	30.	22.	99-102
5.	30.	22.	100-102
6.	30.	22.	100-102
7.	60.	44.5	99-101
8.	60.	45.	100-102
9.	60.	44.	100-101
10.	60.	46.	100-102

An analysis was run on No. 3 above:

ANALYSIS: Calc. for $C_6H_5O_2Br$: Br: 42.33

Found Br: 42.53

(2) PREPARATION OF DIBROMOFLUORESCEIN:

From 4 - Bromoresorcinol:

(a) This compound was first prepared following the method of Kolthoff, Lauer, and Sunde [23] used for the

preparation of dichlorofluorescein. 7.8 g. of phthalic anhydride mixed intimately with 20. g. of 4-bromo-resorcinol was heated to 160°C. in a beaker by means of an oil-bath. 5. g. of anhydrous zinc chloride, finely ground, was then added with stirring during a period of about ten minutes. The temperature was then raised to 180°C. and maintained for two and one-half hours. After cooling, the reaction product was ground and then boiled for twenty minutes in dilute hydrochloric acid (10 cc. conc. acid to 200 cc. water) and filtered. The precipitate was dissolved at room temperature in 3200 cc. of a saturated aqueous solution of sodium bicarbonate, filtered, heated to boiling, and then acidified with approximately 4 normal hydrochloric acid. It was cooled and filtered. The crude yield was 5. g. The diacetate was prepared, and had a melting point of 131 - 160°C., but was very unsatisfactory.

(b) Dibromofluorescein was next prepared using exactly the same procedure as in (a), except that the temperature was held at 180°C. throughout, following

Orndorff and Hemmer's modification for the preparation of fluorescein. 16.4 g. of phthalic anhydride, 42. g. of 4-bromoresorcinol, and 10. g. of anhydrous zinc chloride were used in this case and the yield was 23 g. The diacetate was prepared as in (a) by refluxing 23 g. of the dibromofluorescein with 125 cc. af acetic anhydride and four drops of sulfuric acid for one hour, then pouring into water to precipitate the diacetate. This had a melting point of 122-212°C. after recrystallizing twice for acetic anhydride, and was likewise very unsatisfactory. It was found after solubility tests that 1-4 dioxane was a good solvent for the diacetate, so the diacetate above was recrystallized eight times from 1-4 dioxane until it had a constant melting point. The diacetate was dissolved in the minimum amount of hot 1-4 dioxane and filtered. The filtrate was heated again to boiling and water was added until the first turbidity developed. It was cooled and filtered, yielding a diacetate with a melting point of 214-215°C.

An analysis was run on the diacetate.

ANALYSIS: Calc. for $C_{24}H_{14}O_7Br_2$: Br: 27.87

Found Br: 16.34

The value 16.34 corresponds very closely to the value calculated for a monobromofluorescein diacetate which is calculated to be 16.17, and hence suggests the possibility of a monobromo compound being formed in the fusion with phthalic anhydride.

(c) Dibromofluorescein was next prepared using exactly the same procedure as in (b) except that the time of fusion was increased from two and one-half hours to six and one-half hours. 66.5 g. of phthalic anhydride, 170 g. of 4-bromoresorcinol, and 35 g. of anhydrous zinc chloride were used in this preparation and the yield was 75 g.

A diacetate of this compound was prepared by refluxing 20 g. of the material with 125 cc. of acetic anhydride for six and one-half hours. The reaction product was poured into an excess of ethyl alcohol and the diacetate crystallized out. It was filtered off and

dried, and had a melting point of 194-199°C. The diacetate was recrystallized from 1-4 dioxane as in (b) but a constant melting point could not be obtained, the final melting point being 202-210°C.

A dibenzoate of this dibromofluorescein was also prepared by refluxing 20 g. of the material with 125 cc. of benzoyl chloride for five hours. The reaction product was poured into an excess of ethyl alcohol and a gummy black precipitate settled out. This was filtered off and the filtrate was evaporated to small volume when the dibenzoate crystallized out. The melting point of this dibenzoate was 224-234°C. On recrystallization from benzene, a compound melting at 272-273°C. was obtained, which suggested a tetrabromofluorescein dibenzoate having been formed.

(d) Dibromofluorescein was finally prepared from 4-bromoresorcinol using the same procedure as in (b) except that the temperature was held at 210°C. throughout, and the time of fusion was increased to four hours. 7.3 g. of phthalic anhydride, 20 g. of 4-bromoresorcinol, and 5 g. of anhydrous zinc chloride were used

in this run and the yield was 10 g.

A diacetate was prepared as in (c) and had a melting point of 193-207°C. after two recrystallizations from acetic anhydride and excess ethyl alcohol.

From direct bromination of fluorescein:

(a) 60.5 g. of fluorescein was placed in 240 cc. of 80% acetic acid and warmed to 80°C. 58.7 g. of bromine was dissolved in 195 cc. of 80% acetic acid and was added drop by drop to the fluorescein solution, with vigorous stirring. The stirring was continued for two hours after the reaction was complete, then the dibromofluorescein was filtered off and washed thoroughly with hot water. It had a melting point when dried of 260-264°C.

A diacetate of this dibromofluorescein was prepared by refluxing 20 g. of the material with 125 cc. of acetic anhydride for six and one-half hours. The reaction product was poured into an excess of ethyl alcohol and the diacetate crystallized out. It was

filtered off and dried and had a melting point of 207-209°C. The diacetate was recrystallized by dissolving in the least amount of acetic anhydride, filtering, and pouring into an excess of ethyl alcohol, but a constant melting point could not be obtained. The final melting point was about 270°C. which suggests again that a tetrabromofluorescein diacetate has probably been formed.

A dibenzoate of this dibromofluorescein was also prepared by refluxing 20 g. of the material with 125 cc. of benzoyl chloride for five hours. The reaction product was poured into an excess of ethyl alcohol and the dibenzoate crystallized out on standing. The melting point of this dibenzoate was 228-233°C. On recrystallizing from benzene, a dibenzoate was obtained which melted at 238-238.5°C., which checked with the melting point of the dibenzoate of dibromofluorescein obtained by Orndorff and Hemmer.

(b) Dibromofluorescein was prepared in exactly the same manner as in (a) except that the bromine solution was added all at once instead of drop by drop.

This procedure gave a compound which melted at 283-285°C.

A diacetate of this compound was prepared by refluxing 10 g. of the material with 125 cc. of acetic anhydride for six hours. The reaction product was poured into an excess of ethyl alcohol and the diacetate separated out. This diacetate when recrystallized three times from acetic anhydride and ethyl alcohol gave a constant melting point of 228-229°C.

From bromination of oxonium salt of Fluorescein:

(a) Ten g. of fluorescein was completely brought into solution in hot glacial acetic acid and concentrated hydrochloric acid. 9.6 g. of bromine, in solution in 80 cc. of glacial acetic acid, was added drop by drop with vigorous stirring to the hot solution. The oxonium salt was filtered off and boiled for fifteen minutes with three litres of water to hydrolyse it, then it was filtered and washed thoroughly with hot water. The dibromofluorescein obtained was dried in the oven overnight and had a melting point of 283-291°C. The yield was 12 g.

A diacetate of this compound was prepared by refluxing 12 g. of the material with 125 cc. of acetic anhydride for six hours. The reaction product was poured into an excess of ethyl alcohol and the diacetate separated out. The diacetate when recrystallized four times from acetic anhydride and ethyl alcohol gave a constant melting point of 228-229°C.

ANALYSIS: Calc. for $C_{24}H_{14}O_7Br_2$: Br: 27.87

Found Br: 27.99

A mixed melting point was run on a 50% mixture of this diacetate with the diacetate melting at 228-229°C. obtained from the direct bromination of fluorescein above, and no change could be detected.

(b) The procedure in (a) was repeated using 20 g. of fluorescein and 19.2 g. of bromine to give a yield of 30 g. of dibromofluorescein melting at 278-280°C. The diacetate was prepared, which after three recrystallizations from acetic anhydride and ethyl alcohol gave a melting point of 227-228°C. The yield of the diacetate was 17 g. The diacetate was then hydrolysed

by placing it in 400 cc. of 85% sulfuric acid solution and heating on a water-bath for one and three-quarter hours. The solution was then poured into two litres of water, boiled for fifteen minutes, filtered, and thoroughly washed with hot water. The dibromofluorescein obtained when dried overnight in the oven gave a melting point of 285°C.

(3) ALKALI FUSIONS;

Ten g. of dibromofluorescein from the direct bromination of fluorescein according to Phillips' method was refluxed for two hours with 40 g. of 50% sodium hydroxide solution. The color of the reaction mixture changed from a deep red to a dirty green. 300 cc. of water was added and the solution was acidified with hydrochloric acid. The solution was filtered twice by ordinary filtration and the filtrate was extracted three times with benzene, using three 100 cc. portions. The benzene was allowed to evaporate at room temperature, and the residue was sublimed on a hot-plate and recovered on the inside of a large beaker . The compound

took the form of long needles with a melting point of 102-102.5°C.

A mixed melting point was run on a 50% mixture of this fusion product and 4-bromoresorcinol (melting point 101°C.) previously prepared, and was found to be 72-94°C. This led to the conclusion that 2-bromo-resorcinol is the fusion product which was obtained.

The procedure was repeated three times, and the results in every case proved to be the same.

NOTE :-

All melting points in PART (2) of this thesis are uncorrected as reported, as this seems to be the customary procedure in the literature for this type of work.

SUMMARY.

1. The preparation of dibromofluorescein is best carried out by a method making use of the bromination of an oxonium salt of fluorescein.

2. A diacetate of dibromofluorescein, melting at 228-229°C., has been prepared.

3. Alkali fusions have shown that the bromine atoms in dibromofluorescein are undoubtedly in the 4,5 positions in the fluorescein molecule.

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